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IN RE APPLICATION OF

Art Unit: 1652

CHEN ET AL.

Examiner: MOORE, WILLIAM W

APPLICATION NO: 09/993,180

FILED: NOVEMBER 14, 2001

FOR: POLYNUCLEOTIDE ENCODING A NOVEL HUMAN SERPIN
SECRETED FROM LYMPHOID CELLS, LSI-01

Assistant Commissioner for Patents

Washington, D.C. 20231

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Sir:

1. I, John N. Feder, Ph.D., am an applicant of the patent application Serial No. 09/993,180 identified above and co-inventor of the subject matter described and claimed in this patent application.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit A**.

3. I have performed an experiment, or directed or caused an experiment to be performed, to provide additional evidence that the claimed LSI-01 polynucleotides are useful in the diagnosis of testicular cancers. This experiment demonstrates, unequivocally, that, LSI-01, a polynucleotide of the subject U.S. patent application, is differentially expressed in testicular cancers relative to normal testicular tissue. The results of this experiment support the original teachings of the subject application that the LSI-01 polynucleotides are useful for diagnosing testicular cancers.

Comparison of the expression profiles obtained from normal and diseased tissues is a common method of associating the expression and/or misexpression of a protein to a specific disease and/or disorder.

In this experiment, total RNA from normal testicular tissue and three testicular tumor tissues was isolated using the TriZol protocol (Invitrogen) and quantified by determining its absorbance at 260nm. For comparison purposes, total RNA was also isolated from a number of other tissue and/or tumor types as well according to the same procedure. An assessment of the 18s and 28s ribosomal RNA bands was made by denaturing gel electrophoresis to determine RNA integrity.

The specific LSI-01 sequence to be measured was aligned with related genes found in GenBank to identify regions of significant sequence divergence to maximize primer and probe specificity. Gene-specific primers and probes were designed using the ABI primer express software to amplify small amplicons (150 base pairs or less) to maximize the likelihood that the primers function at 100% efficiency. All primer/probe sequences were searched against Public Genbank databases to ensure target specificity. Primers and probes were obtained from ABI.

For LSI-01, the primer probe sequences were as follows

| | |
|----------------|-----------------------------------|
| Forward Primer | 5'- CCCCTGCCTCACAGGTGTAT -3' |
| Reverse Primer | 5'- CAAAACCAGCCTGCGGTATAG -3' |
| TaqMan Probe | 5' – CCTCAACACCGACTTTGCCTTCCG -3' |

DNA contamination

To access the level of contaminating genomic DNA in the RNA, the RNA was divided into 2 aliquots and one-half was treated with Rnase-free Dnase (Invitrogen). Samples from both the Dnase-treated and non-treated were then subjected to reverse transcription reactions with (RT+) and without (RT-) the presence of reverse transcriptase. TaqMan assays were carried out with LSI-01-specific primers (see above) and the contribution of genomic DNA to the signal detected was evaluated by comparing the threshold cycles obtained with the RT+/RT- non-Dnase treated RNA to that on the RT+/RT- Dnase treated RNA. The amount of signal contributed by genomic DNA in the Dnased RT- RNA was determined to be less than 10% of that obtained with Dnased RT+ RNA. RNA not meeting this threshold was not used in this experiment.

Reverse Transcription reaction and Sequence Detection

100ng of Dnase-treated total RNA was annealed to 2.5 μ M of the LSI-01-specific reverse primer in the presence of 5.5 mM Magnesium Chloride by heating the sample to 72°C for 2 min and then cooling to 55° C for 30 min. 1.25 U/ μ l of MuLv reverse transcriptase and 500 μ M of each dNTP was added to the reaction and the tube was incubated at 37° C for 30 min. The sample was then heated to 90°C for 5 min to denature the enzyme.

Quantitative sequence detection was carried out on an ABI PRISM 7700 by adding to the reverse transcribed reaction the following: 2.5µM forward and reverse LSI-01 primers (see above), 500µM of each dNTP, buffer and 5U AmpliTaq Gold™. The PCR reaction was then held at 94°C for 12 min, followed by 40 cycles of 94° C for 15 sec and 60° C for 30 sec.

Data handling

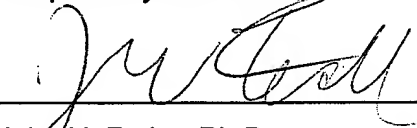
The threshold cycle (Ct) of the lowest expressing tissue (i.e., the highest Ct value) was used as the baseline of expression and all other tissues were expressed as the relative abundance to that tissue by calculating the difference in Ct value between the baseline and the other tissues and using it as the exponent in $2^{(\Delta Ct)}$. In this case, the isolated RNA for the normal testicular tissue and the three testicular tumor tissues, were all on the same plate and were quantified to ensure the total RNA in each well was identical for each tissue.

The results of this experiment are presented in **Exhibit B**. **Exhibit B** represents a comparison between the expression profiles observed in testicular normal tissue and the expression profiles observed in testicular tumor tissue. The data represented in **Exhibit B** was derived from the same tissue expression plate, as discussed above, and hence was part of the same experiment.

4. The following comments relate to **Exhibit B**. To demonstrate the utility of LSI-01 polynucleotides in the diagnosis of testicular cancers, the differential expression pattern of LSI-01 transcripts in normal testicular tissue and three testicular tumor tissues was assessed. As shown in **Exhibit B**, the expression profiling data unambiguously demonstrates that LSI-01 is differentially expressed in testicular tumor tissue relative to normal testicular tissue. LSI-01 transcripts were expressed in testicular tumors at a level that was nearly 10 times greater than the observed expression in normal testicular tissue. This data clearly confirms the utility of using LSI-01 expression as a diagnostic marker for testicular cancers.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,



John N. Feder, Ph.D.

10 / 15 / 2002

Date

Enclosures: Exhibits A, and B.

Exhibit A



Curriculum Vitae

John Nathan Feder, Ph.D.
Bristol-Myers Squibb
Pharmaceutical Research Institute
P. O. Box 5400
Princeton, N. J. 08543

Education

1990 Ph.D. Molecular Biology, Stanford University, Stanford, CA
1980 M.A. Biology, California State University, Chico, CA
1977 B.A. Biology/Chemistry, California State University, Chico, CA

Professional Experience

March 2002 to Present

Bristol-Myers Squibb, Group Leader

Head the Genomic Technologies Group (14 individuals) within the Department of Applied Genomics which includes, DNA sequencing, Affymetrix, micro-array and qPCR platform expression profiling, high-through-put cloning, and functional characterization of orphan GPCRs. Manage external commercial and academic alliances. Participate in exploratory working groups to see that genomic technologies are used efficiently in drug development.

June 1999 to March 2002

Bristol-Myers Squibb, Senior Research Investigator II

Managing 3 individuals in various high-through-put molecular biology and transcriptional profiling applications in support of the Company's Target Class Initiative. Overseeing the Institute's Core and high-through-put sequencing operations. Manage an external alliance (Pharmagene)

March 1999-April 1999

Exelixis, Inc. (Temporary position), Senior Scientist, Constructing full length and EST libraries for the Company's Ag-Bio Program.

January 1999 to March 1999.

Progenitor, Inc. Consultant, Participated in the liquidation of Company's scientific assets.

1997 to Dec 1998

Progenitor, Senior Scientist. Managed the Gene Discovery Group of six individuals and the Hemochromatosis Functional Genomics Group of 2 individuals.

Exhibit A

1993-1997

Mercator Genetics, Scientist I- II. Gene Discovery and Hemochromatosis Functional Genomics. Using physical and genetic information developed at the Company, successfully guided a team of eight individuals to the cloning and the functional characterization of gene responsible for hereditary hemochromatosis, HFE.

1990-1993

Post Doctoral Fellowship. American Cancer Society Fellow, Laboratory of Y.N. and L.Y. Jan. Howard Hughes Institute and Department of Physiology and Biochemistry, University of California, San Francisco, San Francisco, CA. Completed Research lead to the cloning of the mammalian homologs for the Drosophila genes, hairy and numb.

1989

Teaching Assistant, Department of Biological Sciences, Stanford University, Stanford, CA. Taught an upper division class on eukaryotic mechanisms of gene regulation for Dr. R.T. Schimke.

1987

Teaching Assistant, Department of Biological Sciences, Stanford University, Stanford, CA. Taught laboratory classes to Biology majors.

1980-1984

Research Assistant, Laboratory of L.L. Cavalli-Sforza, Department of Genetics, Stanford University School of Medicine, Stanford, CA. As a pre-doctoral research assistant, research completed lead to the publication of eight articles on RFLP mapping in the human genome.

1980

Lecturer, Department of Chemistry, California State University, Chico. Taught Organic Chemistry to non-majors.

Publications

Ning, L., Chen, S., Wu, S., Sun, L., Huang, M., Levesque, P. C. Rich, A., Feder, J. N. and M. Blonar. 2003 Expression and characterization of human transient receptor potential melastatin 3(hTRPM3). J. Biol. Chem. 278:20890-20897

Cheng, D., Nelson, T. C., Chen, J., Meegalla, R., Taub, R., Billheimer, J. T., Ramaker, M. and J. N. Feder. 2003 Identification of Acyl Coenzyme A: Monoacylglycerol Acyltransferase 2, an intestine specific enzyme implied in dietary fat absorption. J. Biol. Chem. 278:13611-13614

Roy, C. N., E. J. Carlson, E. L., Anderson, A. Irrinki, S. M, Starnes, Feder, J. N. and C. A. Enns. 2000. Interactions of the Ectodomain of HFE with the transferrin receptor are critical for iron homeostasis in cells. FEBS Lett. 484 :271-274.

Roy, C. N., D. Penny, J, N. Feder and C. A. Enns. 1999. The hereditary hemochromatosis protein, HFE specifically regulates Tf-mediated iron uptake in HeLa cells. J. Biol. Chem. 274: 9022-9028.

Exhibit A

- Tsuchihasni, Z., S. L. Hansen, L. Quintana, G J. N. Feder, R. K. Wolff. 1998. Transferrin receptor mutation analysis. S. Kronmal, F. A. Mapa, in hereditary hemochromatosis patients. Blood Cells, Molecules and Diseases 24: 317-321.
- Gross, C. N., A. Irrinki, J. N. Feder and C. E. Enns. 1998. Co-trafficking of HFE with transferrin receptor implies role in intracellular iron regulation. J. Biol. Chem. 273: 22068-22074.
- Lebron, J. A., M. J. Bennett, A. J. Chirino, D. E. Vaughan, P. M. Snow, G. A. Mintier, J. N. Feder, and P. J. Bjorkman. 1998. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 93:111-123.
- Feder, J. N., D. M. Penny, A. Irrinki, V. K. Lee, et al. 1998. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. PNAS 95: 1472-1477.
- Waheed, A., S. Parkkila, X.Y. Zhou, S. Tomatsu, Z. Tsuchihashi, J.N. Feder, et al. 1997. HFE mutations in hereditary hemochromatosis: Effects on association with β_2 -Microglobulin, intracellular transport, and cell surface delivery of the HFE protein in transfected COS-7 cells. PNAS 94: 12384-12389.
- Feder, J.N., Z. Tsuchihashi, A. Basava, et al. 1997. The hemochromatosis founder mutation in HLA-H disrupts β_2 -Microglobulin interaction and cell surface expression. J. Biol. Chem. 272: 14025-14028.
- Chen, H., A. Thiagalingam, H. Chopra, W. Borges, J.N. Feder, B.D. Nelkin, B.S. Baylin, and D.W. Ball. 1997. Conservation of the Drosophila lateral inhibition pathway in human lung cancer: a hairy-related protein (HES-1) directly represses achaete-scute homolog-1 expression. PNAS 94: 5355-5360.
- Ruddy, D.A., V.K. Lee, G.S. Kronmal, G.A. Minitier, L. Quintana, R. Domingo, N.C. Meyer, A. Basava, E. McClelland, A. Fullan, F.A. Mapa, T. Moore, W. Thomas, D.B. Loeb, C. Harmon, Z. Tsuchihashi, R.K. Wolff, R.C. Schatzman, and J.N. Feder. 1997. A 1.1 megabase transcript map of the hereditary hemochromatosis locus. Genome Research. 7: 441-456.
- Parkkila, S., A. Waheed, R.S. Britton, J.N. Feder, Z. Tsuchihashi, R.C. Schatzman, B.R. Bacon, and W.S. Sly. 1997. Immunohistochemistry of HLA-H, the protein defective in patients with hereditary hemochromatosis, reveals unique pattern of expression in gastrointestinal tract. PNAS. 94: 2534-2539.
- Feder, J.N., A. Gnirke, W. Thomas, et al., 1996. A novel MHC class I-like gene mutated in patients with hereditary haemochromatosis. Nature Genetics 13: 399-408.
- Zhong, W., J.N. Feder, M. Jiang, L Y.Jan, and Y.N.Jan. 1996. Asymmetric Localization of a mammalian numb homolog during mouse cortical neurogenesis. Neuron 17: 43-53.

Exhibit A

- Feder, J.N., L. Li, L Y. Jan, and Y.N. Jan. 1994. Genomic cloning and chromosomal location of the human homolog to the Drosophila segmentation gene, hairy. *Genomics* 20: 56-61.
- Feder, J.N., L Y. Jan, and Y.N. Jan. 1993. A rat gene with sequence homology to the Drosophila gene hairy is rapidly induced by growth factors known to influence neuronal differentiation. *Mol. Cell. Biol.* 13: 105-113.
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- Spurr, N.K., D. Kelseli, L. Rooke, L.L. Cavalli-Sforza, A. Bowcock, and J. Feder. 1991. Assignment of eight human genomic DNA sequences detecting high frequency RFLPs. *Ann. Hum. Genet.* 55: 147-150.
- Feder, J.N., C.J. Guidos, B. Kusler, C. Carswell, D. Lewis, and R.T. Shimke. 1990. A cell cycle analysis of growth-related genes expressed during T lymphocyte maturation. *J. Cell Biol.* 111: 2693-2701.
- Feder, J.N., Y.G. Assaraf, L.C. Seamer, and R.T. Shimke. 1989. The pattern of dihydrofolate reductase expression through the cell cycle in rodent and human cultured cells. *J. Biol. Chem.* 264: 20583-20590.
- Feder, J.N., A. Bowcock, L.L. Cavalli-Sforza, and R.T. Shimke. 1987. Two RFLPs for the human dihydrofolate reductase gene (DHFR). *Nucl. Acid Res.* 15: 5906.
- Johnston, R.N., J. Feder, A.B. Hill, S.W. Sherwood, and R.T. Shimke. 1986. Transient inhibition of DNA synthesis results in increased dihydrofolate reductase synthesis and subsequent increased DNA content per cell. *Mol. Cell. Biol.* 6: 3373-3381.
- Spurr, N.K., J. Feder, W.F. Bodmer, P.N. Goodfellow, E. Solomon, and L.L. Cavalli-Sforza. 1986. Chromosomal assignment of seven human genomic DNA sequences associated with restriction fragment length polymorphisms. *Ann. Hum. Genet.* 50: 145-152.
- Feder, J., L. Yen, E. Wijsman, L. Wang, L. Wilkins, J. Schroder, N. Spurr, H. Cann, M. Blumenberg, and L.L. Cavalli-Sforza. 1985. A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. *Am. J. Hum. Genet.* 37: 635-649.
- Ohlsson, M., J. Feder, L.L. Cavalli-Sforza, and A. von Gabain. 1985. Close linkage of β and α interferons and infrequent duplication of B interferon in humans. *Proc. Natl. Acad. Sci. USA.* 82: 4437-4476.

Exhibit A

Feder, J., H.M.D. Gurling, J. Darby and L.L. Cavalli-Sforza. 1985. DNA restriction analysis of the proopiomelanocortin gene in schizophrenia and bipolar disorders. *Am. J. Hum. Genet.* 37: 286-294.

Darby, I.K., J. Feder, M. Selby, V. Riccardi, K. Ferrell, D. Sian, K. Goslin, W. Rutter, E.M. Shooter and L.L. Cavalli-Sforza. 1985. A discordant sibship analysis between β -NGF and neurofibromatosis. *Am. J. Hum. Genet.* 35: 52-59.

Feder, J., N. Migone, A.C.Y. Chang, M. Cochet, S.N. Cohen, H. Cann and L.L. Cavalli-Sforza. 1983. A DNA polymorphism in close physical linkage with the proopiomelanocortin gene. *Am. J. Hum. Genet.* 35: 1090-1096.

Migone, N., J. Feder, H. Cann, B. Van West, J. Hwang, N. Takahasi, T. Honjo, A. Piazza and L. L. Cavalli-Sforza. 1983. Multiple DNA fragment polymorphisms associated with immunoglobulin μ -chain switch-like regions in man. *Proc. Natl. Acad. Sci. USA.* 80: 467-471.

Book Chapters and Invited Reviews

- 1) Feder, J. N. 1999. Review- The hereditary hemochromatosis gene (HFE): a MHC class I-like protein that functions in the regulation of iron homeostasis. *Immunologic Research*, 20:175-185.
- 3). Gallie, D.R., J.N. Feder, and V. Walbot. 1992. GUS as a useful reporter in animal cells. In: *GUS protocols*, pp. 181-188. Ed. Sean R. Gallager. Academic Press, Inc.
- 4). Schimke, R.T., S. Sherwood, R. Johnston, A. Hill, G. Rice, C. Hoy, J. Feder, and P. Farnham. (1988) On the mechanism of induced gene amplification in mammalian cells. In: *Mechanism of Drug Resistance in Neoplastic Cells*. Bristol-Meyers Cancer Symposia. Vol. 9. pp 29-40. Eds. Woolley P.V. and Tew, K.D. Academic Press, Inc.

Invited Talks (since 1996)

October 1996, 6th international workshop on the identification of transcribed sequences, Edinburgh, Scotland, Title: " Transcript map surrounding the hemochromatosis candidate gene, reveals several non-MHC gene families"

January 1997, Cambridge Healthtech Institute, Novel target identification for drug discovery: Genomic Approaches, San Diego, CA. Title: "Positional cloning of a novel MHC class I-like sequence, a strong candidate gene for hereditary hemochromatosis"

Exhibit A

February 1997, University of California, San Francisco, Department of Gastroenterology, Title: "Positional cloning of the hereditary hemochromatosis gene"

March 1997, California State University, Chico, Department of Chemistry, Title: "Positional cloning of the hereditary hemochromatosis gene"

November 1997, University of Texas, Southwestern Medical School, Department of Cell Biology and Neurosciences. Title: "Cloning of the hereditary hemochromatosis gene and analysis of the gene product"

December 1997, Beckman Research Institute of the City of Hope, Title: The Molecular biology of hemochromatosis"

May 1998, NIDDK symposium on molecular medicine and hemochromatosis: At the crossroads. Bethesda, MD. Title: "The HFE gene"

June 1998, International conference on the Molecular Biology of Hematopoiesis, Borimo, Italy. Title: "The molecular biology of the HFE gene"

October 1998, Hemochromatosis Foundation, Hershey, Pennsylvania, Title: "The discovery of the HFE gene and its function"

December 1998, University of Wisconsin, Madison, Biochemistry Department, Title: "The hereditary hemochromatosis gene product (HFE) and cellular iron homeostasis"

February 1999, Albert Einstein University, Department of physiology and biophysics, New York, Title: "The hereditary hemochromatosis gene product (HFE) and cellular iron homeostasis"

February 1999, Harvard University Medical School, Title: "The hereditary hemochromatosis gene product (HFE) and cellular iron homeostasis"

March 1999, Oregon Health Sciences University, Portland, Oregon Title "The hereditary hemochromatosis gene product (HFE) and cellular iron homeostasis"

May 1999, Co-Chaired the session on the Hemochromatosis gene, World Congress on iron metabolism, Serrento, Italy

September 2000 German national meeting of gastroenterology, Title Hemochromatosis. Hamburg, Germany

Awards and Honors

2002-2004 Recipient of 5 innovation awards at Bristol-Myers Squibb

1998

Member of Advisory Council for the Chemistry Department at California State University, Chico.

1998

Member of the Iron Disorders Institute Scientific Review Board.

Exhibit A

1997

Co-recipient of the Marcel Simon Award for achievement in the field of iron storage disease.

1990-93

Recipient of an American Cancer Society Post-Doctoral Fellowship.

1990

Recipient of a NIH Post-Doctoral training grant.

Patents (issued)

US6284732 B1

Peptides and peptide analogues designed from HFE protein and their uses in the treatment of iron overload diseases. Bio-Rad Laboratories, Inc.

Inventor(s): Feder, John N.; Schatzman, Randall C. ; Bjorkman, Pamela J. ; Bennett, Melanie ; Lebron, Jose

Application No. 09/216077, Filed 19981218, Issued 20010904 Granted 20010904

US6228594 B1

Method for determining the presence or absence of a hereditary hemochromatosis (^) gene mutation

Bio-Rad Laboratories, Inc.

Inventor(s): Thomas, Winston J. ; Drayna, Dennis T. ; Feder, John N. ; Gnirke, Andreas ; Ruddy, David ; Tsuchihashi, Zenta ; Wolff, Roger K.

Application No. 09/503444, Filed 20000214, Issued 20010508 Granted 20010508

US6140305 A1

Hereditary hemochromatosis gene products

Bio-Rad Laboratories, Inc.

Inventor(s): Thomas, Winston J. ; Drayna, Dennis T. ; Feder, John N. ; Gnirke, Andreas ; Ruddy, David ; Tsuchihashi, Zenta ; Wolff, Roger K.

Application No. 834497, Filed 19970404, Issued 20001031

US6025130 A1

Hereditary hemochromatosis gene

Mercator Genetics, Inc.

Inventor(s): Thomas, Winston J. ; Drayna, Dennis T. ; Feder, John N. ; Gnirke, Andreas ; Ruddy, David ; Tsuchihashi, Zenta ; Wolff, Roger K.

Application No. 652265, Filed 19960523, Issued 20000215

US5753438 A1

Method to diagnose hereditary hemochromatosis

Mercator Genetics, Inc.

Inventor(s): Drayna, Dennis T. ; Feder, John N. ; Gnirke, Andreas ; Kimmel, Bruce E. ; Thomas, Winston J. ; Wolff, Roger K.

Application No. 436074, Filed 19950508, Issued 19980519

US5712098 A1

Hereditary hemochromatosis diagnostic markers and diagnostic methods

Exhibit A

Mercator Genetics

Inventor(s): Tsuchihashi, Zenta ; Gnirke, Andreas ; Thomas, Winston J. ; Drayna, Dennis T. ; Ruddy, David ; Wolff, Roger K. ; Feder, John N.
Application No. 632673, Filed 19960416, Issued 19980127

S5705343 A1

Method to diagnose hereditary hemochromatosis

Mercator Genetics, Inc.

Inventor(s): Drayna, Dennis T. ; Feder, John N. ; Gnirke, Andreas ; Kimmel, Bruce E. ; Thomas, Winston J. ; Wolff, Roger K.

Application No. 599252, Filed 19960209, Issued 19980106

US20030082782 A1

Polynucleotides encoding a novel metalloprotease, MP-1

Inventor (s) Chen, Jian ; Feder, John N. ; Nelson, Thomas C. ; Krystek, Stanley R. ; Duclos, Franck

Application Number: 10/067443

US6706513 B2

Adenosine deaminase homolog

Inventor(s) Feder, John N. ; Ramanathan, Chandra S. ; Mintier, Gabe

Application Number: 09/933386

Additional Patent applications filed

Title: ACETYL COA CARBOXYLASE 2 SEQUENCES AND METHODS

Inventors: Dong Cheng; John Feder; Ching-Hsuen Chu; Luping Chen

Filed: July 23, 2004.

Over 100 target class gene patents, including 30 orphan GPCRs including functional data

Peer Review for the following Journals:

Nature Genetics

Analytical Biochemistry

Lancet

Science

Genome Research

Genomics

American Journal of Human Genetics

Journal of Laboratory and Clinical Medicine

Diabetologia

Biochemical Genetics

Journal of Biological Chemistry

Molecular Medicine Today

Journal of Rheumatology

References

Available upon request



Exhibit B

Relative Expression of LSI-01 In testicular Tumors and Normal Control RNA

